Consommation et Affaires commerciales Canada
Bureau des brevets
Ottawa, Canada
K1A 0C9

Consumer and Corporate Affairs Canada
Patent Office

(21) (A1) 2,081,119
(22) 1991/04/25
(43) 1991/10/28

5,029,8/16

(51) INTL.CL.[5] A61K-009/127; A61K-047/24

(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Liposomes with an Excess Positive Charge

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(30) (DE) P 40 13 632.9 1990/04/27

(57) 11 Claims

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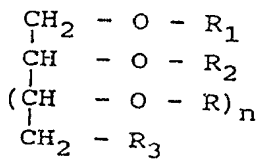
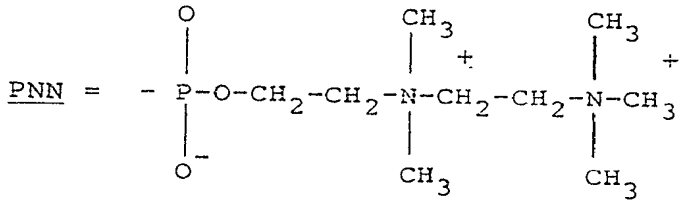
Abstract

New liposome structures are described which are characterized in that they contain at least 1 mol-% of a compound having the general formula (I) with an excess positive charge



(I)

in which
R_1 denotes alkoyl or alkyl each with 14 to 18 C atoms, oleoyl or oleyl,
R_2 denotes the group PNN



R_3 = $-O-R_1$, $-O-PNN$, $-O-Gly$, NH_3^+, $NH_2CH_3^+$, $NH(CH_3)_2^+$ or $N(CH_3)_3^+$,
R has one of the meanings stated for R_1, R_2 or R_3 and n denotes a whole number from 0 to 3, and preferably denotes 0, provided that the molecule contains one of the said groups with a positive charge.

Due to their organ-specific characteristics the liposomes according to the present invention are especially suitable as pharmaceutical carriers in a pharmaceutical preparation for the treatment of diseases in the liver organ which contains one or several active substances which are active in the liver enclosed in the liposomes according to the present invention.

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Description

The invention concerns liposomes with an excess positive charge which can be used as carriers of active substances when treating liver diseases.

Liposomes are spherical structures consisting of one or several lipid double layers with an aqueous inner cavity (lipid vesicles). These vesicles can be produced by mechanical fine dispersion of phospholipids (e.g. lecithin) in aqueous media.

Bangham et al., J. Mol. Biol. 13 (1965) 238-252 observed that phospholipids form superstructures in the presence of water. Depending on physical parameters such as pressure, temperature and ion concentration formation of micelles, unilamellar or multilamellar liposomes or even simple lipid double layers occurs (cf. Liposomes: From physical structure to therapeutic application (1981), Knight, C.G. (Ed.), Elsevier, North Holland Biomedical Press, chapter 2: H. Eibl, Phospholipid synthesis, 19-50; chapter 3: F. Szoka and D. Papahadjopoulos, Liposomes: Preparation and characterization, 51-104). Small unilamellar liposomes are spherical structures with a diameter of 20 to 200 nm (cf. Barenholtz et al., FEBS Lett. 99 (1979) 210-214). Their inner volume consists of water which is delimited towards the outside by a lipid double layer. Depending on the lipophilicity or hydrophilicity, active substances can either be enclosed within the lipid double layer or in the aqueous inner volume of the liposomes and can then be transported and distributed in the organism via the body fluids.

As a result of their structure liposomes serve as membrane models in biochemistry and molecular biology. In recent years several papers have been published on the properties of liposomes and their use as carriers for pharmaceutical agents (cf. e.g. H. Schreiner and M. Raeder-Schikorr, "Pharmazie in unserer Zeit 11 (1982) 97-108). Animal experiments which have been published up to now show that liver and spleen usually dominate over other organs with regard to liposome uptake. About 8 % of the liposomes are found in the liver after one hour and about 15 % after 24 hours. However, there are only slight differences between liposomes which are neutral and those which carry a 10 % excess negative charge with regard to organ distribution and accumulation in organs.

The main aim of a possible application of liposomes in medical science is to selectively treat diseases. The intention is to promote the desired effects of the active substance enclosed in the liposomes and on the other hand to reduce the undesired effects (improvement of the therapeutic index). Such a concept is convincing in the treatment of an isolated disease which is restricted to a certain organ which is particularly important in the case of liver diseases. Provided that the enclosed active substances only reach the liver and are not distributed unspecifically over the entire organism, the treatment of the diseased organ would be largely specific. The following two examples from the internal medicine elucidate these concepts and show the practical significance of this principle:

Example 1: Malignant liver diseases

When malignant tumours form metastases the liver can be the main site for metastases formation e.g. in the case of breast cancer or in tumours of the gastro-intestinal

tract. However, the liver can also be the only organ where metastases are formed, e.g. in operated colonic cancers which later leads to liver metastases. In contrast to this primary hepatocellular carcinomas are restricted only to the liver.

Example 2: Inflammatory liver diseases

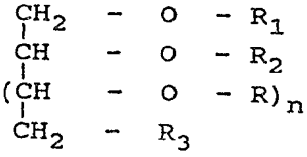
With the introduction of the interferons (Ifn), a group of substances is available for the first time which is promising for the treatment of chronic viral hepatitis. The enclosure of Ifn into liposomes and their quantitative transport into the diseased liver is expected to considerably reduce Ifn-specific side-effects. In addition the time intervals for the Ifn application can probably be considerably increased, it may be possible to reduce the four administration per week to a weekly application.

The object of the present invention is therefore to provide liposomes with which the aforementioned problem of an organ-specific transport of active substance can be solved and with which it is possible to obtain an improvement of the therapeutic index of the active substances enclosed in the liposomes.

This object is achieved using the subject matter of the present invention.

The present invention provides new liposome structures which are characterized in that they contain at least 1 mol-% of a compound having the general formula (I)

which has an excess positive charge

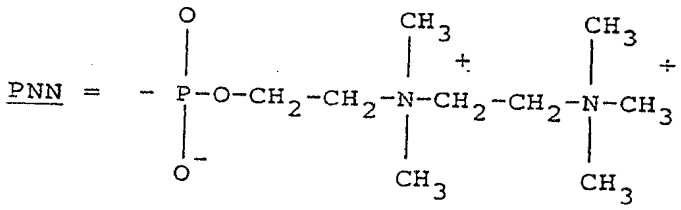


(I)

in which

R_1 denotes alkoyl or alkyl each with 14 to 18 C atoms, oleoyl or oleyl,

R_2 denotes the group PNN



R_3 denotes = -O-R_1, -O-PNN, -O-Gly, NH_3^+, $NH_2CH_3^+$, $NH(CH_3)_2^+$ or $N(CH_3)_3^+$,

R has one of the meanings stated for R_1, R_2 or R_3 and n denotes a whole number from 0 to 3 and preferably 0, provided that the molecule contains one of the said groups having a positive charge.

Phospholipid-like compounds which have a structure which is comparable to the aforementioned formula (I) in which one of the residues comprises R, R_1, R_2 or R_3 PNN and processes for their production are known from DE-A-27 52 553.

It was now surprisingly found that liposomes from lipid mixtures which contain at least 3 mol-% of a compound

having the general formula (I) exhibit organ-specific characteristics as carriers for pharmaceutical agents. The liposomes according to the present invention preferably contain 1 to 30 mol-% and in particular 5 to 15 mol-% of a compound having the general formula (I) whereby the liposomes can also contain mixtures of one or several compounds having the general formula (I).

The following Table 1 shows that for liposomes according to the present invention labelled with [^{3}H] inulin and containing a compound having the general formula (I) which have a positive excess charge the content of [^{3}H] inulin in the blood has already decreased to less than 1 % after one hour. In contrast an extraordinarily high percentage of [^{3}H]-inulin is present in the liver: 67 % after 1 hour, 54 % after 24 hours and still 45 % after 72 hours. Inulin could not be detected in organs such as the lung, kidney, heart and brain. These organs are therefore not included in Table 1. Surprisingly, liposomes could not be found even in the spleen which usually takes-up liposomes well as part of the phagocytosing system. This is elucidated in the following Table 2 in which the [^{3}H]-inulin uptake is shown for blood, liver and spleen per g fresh weight.

Table 1

Comparison of the [^{3}H]-inulin uptake after enclosure in liposomes having different surface charges and after addition of free [^{3}H]-inulin.

Lipids (mol/mol)	Time (h)	Blood (% of the applied amount)	Liver	Spleen
PPGPC/chol	1	59	11	3
60 / 40	24	3	20	5
	72	0	17	2
PPGPC/PPGPG/chol	1	64	7	1
50 / 10 / 40	24	8	16	1
	72	0	11	2
+				
PPGPC/N/chol	1	1	67	2
50 / 10 / 40	24	0	54	0
	72	0	45	0
free [^{3}H]-inulin	1	0.6	0	0
	24	0	0	0

The distribution in the organs is stated as a percentage of the applied amount of inulin. The uptake of [^{3}H]-inulin in NMR-I mice is almost linear over a large dosage range (up to 1 mmol per kg body weight). Free inulin has already left the circulatory system after 1 hour and is completely excreted by the kidney. The various abbreviations denote: h = hour, PPGPC = 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; chol = cholesterol; PPGPG = 1,2-dipalmitoyl-sn-glycero-3-phospho-sn-1-glycerol, sodium salt; N^+ = a positive, double-chained lipid of the general formula (I).

Table 2

Comparison of [^{3}H]-inulin uptake after enclosure in liposomes in percent per g fresh weight. All other details correspond to those in Table 1.

Lipids (mol/mol)	Time (h)	Blood (% of the applied amount per g fresh weight)	Liver	Spleen
PPGPC/chol	1	26	8	19
60 / 40	24	2	15	38
PPGPC/PPGPG/chol	1	35	6	11
50 / 10 / 40	24	4	13	18
PPGPC/N^+/chol	1	0	37	11
50 / 10 / 40	24	0	31	0
free [^{3}H]-inulin	1	0.6	0	0
	24	0	0	0

It is apparent from the values shown in the above Tables 1 and 2 that it is possible to concentrate the active substance exclusively in the liver by using the liposomes according to the present invention comprised of double-chained lipophilic structures and containing a compound having the general formula (I) with an excess positive charge. This surprising fact can be used for the therapy of liver diseases.

In addition the present invention also concerns a pharmaceutical preparation for the treatment of liver

diseases containing one or several active substances against liver diseases which are enclosed in liposomes which is characterized in that the liposomes contain at least 1 mol-%, preferably 1 to 30 mol-% and in particular 5 to 15 mol-%, of compounds having the aforementioned general formula (I) which have an excess positive charge in which R_1, R_2, R_3, R and n have the meanings stated above. The amount of enclosed active substance is limited by the solubility of the active substance in water in the case of water-soluble pharmaceuticals.

A further subject matter is also the use of a compound having the general formula (I) for the production of a pharmaceutical agent for the treatment of liver diseases which contains one or several active substances against diseases in the liver region enclosed in liposomes and in which the liposomes contain at least 1 mol-% of a compound of formula (I).

The lipids of the general formula (I) according to the present invention which can be used to produce liposomes according to the present invention which are to accumulate in the liver are:

Phospholipid-like structures containing the residue PNN which does not occur naturally;

Preferred lipids are:
1,2-MM-sn-G-3-PNN, 1,2-PP-sn-G-3-PNN, 1,2-SS-sn-G-3-PNN, 1,2-TeTe-sn-G-3-PNN, 1,2-HeHe-sn-G-3-PNN and 2,2-OcOc-sn-G-3-PNN; or the corresponding phospholipids based on 1,3-diacyl- or 1,3-dialkylglycerides as starting products.

The lipids used according to the present invention can for example be produced according to methods stated for such compounds in DE-A-27 52 553.

In the aforementioned production processes for compounds of the general formula (I) it is preferable to use the general procedures stated in the examples for the production of compounds according to the present invention under the reaction conditions stated there.

In order to produce the liposomes with an excess positive charge according to the present invention, the individual liposome components, e.g. PPGPC, chol and N^+, in which N^+ represents one or several of the lipids of the general formula (I) according to the present invention, are dissolved in a suitable solvent preferably while heating in order to obtain a homogeneous mixing of the components. The solvent is removed in a vacuum and an aqueous buffer solution is added to the finely dispersed lipid film (all solutions which can be used physiologically can be used as the aqueous buffer solution). Subsequently the mixture is kept at one temperature for ca. 1 hour while gently agitating which is usually about 5°C above the main transition temperature of the lipids e.g. at ca. 50°C.

The pre-heated lipid suspension is then transferred to the pressure chamber of a French press (Amico Company, Silver Spring, USA). The French press consists of a hydraulic press and a standard pressure chamber made of steel with a maximum filling volume of 40 ml. After closing the pressure chamber the pressure is increased to 20000 psi and the liposome dispersion is pressed through a narrow outlet under constant pressure. The process is repeated at least three times. After

centrifuging the liposome dispersion (Sorvall RC-5B: 5°C, 30 minutes at 27000 g) the supernatant is removed from the sediment. It contains the liposomes which are then available for the various applications and investigations e.g. for the production of a pharmaceutical preparation according to the present invention (liposomes containing the active agent). The liposomes according to the present invention can also be produced according to other methods.

The present invention therefore also concerns a process for the production of liposomes according to the present invention which contain at least 3 mol-% of a compound of the general formula (I) with an excess positive charge in which R_1, R_2, R_3, R and n have the aforementioned meanings and which is characterized in that at least 3 mol-% of a compound having the general formula (I) together with the other liposome components which are in an amount which together with the compound having the general formula (I) totals 100 mol-% is converted into a lipid suspension.

For this the compounds of formula (I) according to the present invention are used in an amount of 1 to 30 mol-% and in particular of 5 to 15 mol-% (total liposomes = 100 mol-%).

The following procedure is used to produce a pharmaceutical preparation containing one or several active agents which are enclosed in the liposomes according to the present invention (production of the liposomes containing the active agent):

In order to enclose water-insoluble substances the active agent is dissolved with the lipids in methylene chloride or chloroform; after this one proceeds according to the process described above for the production of empty liposomes according to the present invention.

In order to enclose water-soluble substances a buffer solution is added to the lipid film as described above for the production of empty liposomes which now contain the active water-soluble substance. The subsequent procedure is as described for the production of empty liposomes. After centrifugation the supernatant contains the non-enclosed active water-soluble substance in addition to the filled liposomes. This free portion of the active substance can be separated from the portion enclosed in the liposomes by gel chromatography (cf. Liposomes: From physical structure to therapeutic application (1981), l.c.). It is preferable to concentrate the liposomes by ultrafiltration (Amicon Company or Sartorius). It is expedient to carry out a sterile filtration with membrane filters before using the liposomes (Sartorius Company, pore diameter 0.2 μm).

The present invention therefore also concerns a process for the production of a pharmaceutical preparation which contains one or several active substances which are enclosed in the liposomes according to the present invention which is characterized in that one uses the process for the production of liposomes according to the present invention which contain at least 3 mol-% of a compound having the general formula (I) and in order to enclose active water-insoluble substances the active substance is dissolved together with the lipids and in order to enclose active water-soluble substances an

aqueous buffer solution containing the active water-soluble substance is added to the lipid film.

The active substances should be preferably transported into the liver. One or several active substances are preferably selected, e.g. from the group of cytostatic agents (hexadecyl-phosphocholine, 1-octadecyl-2-methyl-rac-glycero-3-phosphocholine, 5-fluorouracil, epirubicin, adriamycin, cis-platinum complexes, Novantron), from the class of immunomodulating substances (interferon α, MAF = macrophage activating factor), antimycotically active substances (Amphotericin B) and substances active against protozoal diseases (malaria, trypanosomal infections and Leishmania infections).

It is intended to further elucidate the present invention by the following examples without, however, limiting it by them.

If not stated otherwise amounts and percentages are related to weight, and stated temperatures refer to the celsius scale.

Examples

Examples for the production of compounds of the lipid group A according to the present invention:
General procedure:

The procedure for the production of the compounds is

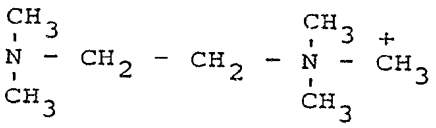
a) to react a compound having the general formula (I) in which R_3 denotes an OH group (denoted a compound of the general formula (II) in the following) with $POCl_3$ in a known manner,

b) to react the reaction product from step a) in a known manner with a compound having the general formula (III)

$$HO - Alk - Hal \qquad (III)$$

in which Hal denotes Cl, Br or I,

c) to react the reaction product from step (b) with a compound having the general formula (IV)



(IV)

Those compounds of the general formula (III) which contain bromine are preferred.

The reaction of phosphoryl chloride with the compound having the general formula (II) in step a) is preferably carried out in an inert organic solvent. Examples of suitable solvents are halogenated hydrocarbons such as chloroform and carbon tetrachloride, aromatic hydrocarbons such as benzene or tolouene and aliphatic hydrocarbons such as petroleum ether and the like. Cyclic organic solvents such as tetrahydrofuran are also suitable. Trichloroethylene and tetrahydrofuran are preferred since the salts which form in this case such as triethylamine hydrochloride have a very low solubility and therefore precipitate and can be easily removed by filtration. Moisture should be excluded as far as possible when carrying out the reaction. Suitable temperatures are in the range of -10 to 50°C, preferably between 10 and 30°C. However in some cases, depending on

the substances and solvents used, temperatures which are above or below this are also employed.

The reaction is preferably carried out in the presence of an inert organic base such as triethylamine, pyridine or quinoline.

It is expedient to dissolve the phosphoryl chloride in the inert solvent and to add the base. Then the compound having the general formula (II) which it is expedient to also dissolve in an inert solvent is added. This is preferably carried out by dropwise addition while stirring. Since the reactions proceed smoothly and unambiguously the temperature can be chosen from case to case so that the reaction is completed immediately after the dropwise addition which can be easily determined by examination by thin layer chromatography.

Step b) proceeds smoothly when the product of step a) is mixed with a compound having the general formula (III). For this a solution of the compound having the general formula (III) is preferably added to the reaction mixture in the presence of an organic base such as triethylamine. The reaction is preferably carried out at temperatures between 20 and 60°C using tetrahydrofuran as the solvent. Depending on the selected conditions the reaction period is generally between 20 and 120 minutes.

Under the preferred conditions the hydrohalogenide of the base precipitates and is removed. In order to achieve the best yields, the hydrochloride is re-washed and the washing liquid is again added to the reaction solution. Then the solvent is removed. The residue is then dissolved in tetrahydofuran, if desired, and

hydrolysed with a weak alkaline aqueous solution, for example sodium bicarbonate in water, during which the pH is preferably kept between 5 and 7. Then it is extracted with an organic solvent such as diisopropyl ether or chloroform. In this way the sodium salts of the alkylphosphatidic acids are obtained which can be readily recrystallized.

The reaction in step c), that is the reaction of the product of step b) with the amino base, is also usually carried out in polar solvents such as chloroform, primary, secondary or tertiary alcohols, dimethylformamide, acetonitrile, nitromethane or water or mixtures thereof. Depending on the sensitivity of the starting substances used, all temperatures between the solidification point and the boiling point of the solvent used or solvent mixture come into consideration for the reaction. The reaction is preferably carried out at temperatures between room temperature and the boiling point of the solvent. Thus at 50°C the reactions are usually completed after 2 to 8 hours. The reaction product is subsequently isolated and can be recrystallized. A chromatographic purification is also possible. The yields are generally over 50 % of the theoretical value in relation to diglyceride as the starting product.

The high yields of the desired product obtained are surprising since with regard to the many functional groups in the reaction partners it was not possible to predict a smooth reaction course in the desired direction.

The steps a), b) and c) in the process mentioned above are preferably carried out as follows:

Step a)

0.04 mol of an alcohol having the general formula (II) - dissolved in 40 ml trichloroethylene and 9 g triethylamine - is added to 10 ml trichloroethylene and 6.6 g $POCl_3$ (0.044 mol) in an ice-bath at 0 to 5°C. If the alcohol is a diacylglycerol, 9 g triethylamine in 10 ml trichloroethylene and then immediately afterwards diacylglycerol in 30 ml trichloroethylene are added successively to the acylation mixture in order to prevent acyl migration. 25 ml toluene are used for re-washing. The ice-bath is replaced by a water bath at 20°C. The reaction is already completed after 20 minutes at 20°C.

Step b)

0.048 mol compound (III), e.g. dissolved in 75 ml tetrahydrofuran and 13 g triethylamine, is added at 20°C to the reaction mixture of step a) and 25 ml tetrahydrofuran are used for re-washing. The reaction comes to completion after 20 minutes at 35°C. It is filtered, washed again in 50 ml toluene and concentrated by evaporation. The hydrolysis is carried out by the successive addition of 30 ml ice water, 30 ml 1 M sodium acetate after 2 minutes and 90 ml tetrahydrofuran after a further 2 minutes. The hydrolysis is completed after 12 hours. There is practically only one product formed.

Step c)

B. 0.04 mol product from step b) (ß-bromoethyl ester) is dissolved in 90 ml $CHCl_3$ and admixed with 150 ml isopropanol as well as with 200 ml 40 % N,N,N,N-tetramethylethylene-diamine in water.

The following compounds No. 1 to 8 are obtained with the process described above:

1) 1,2-dimyristoyl-sn-glycero-3-phospho-(N,N-dimethyl-N-[N',N',N'-trimethyl-ethylammonio])-ethylammonium chloride,
$C_{40}H_{82}ClN_2O_8P$ (785.5)
Identified by a TLC comparison with the 1,2-dipalmitoyl derivative (see substance 2)

2) 1,2-dipalmitoyl-sn-glycero-3-phospho-(N,N-dimethyl-N-[N',N',N'-trimethyl-ethylammonio])-ethylammonium chloride,
$C_{44}H_{90}ClN_2O_8P$ (841.6)
calc. (%): C, 62.79; H, 10.78; N, 3.33; P, 3.68
found (%): 62.54; 10.71; 3.21; 3.67

3) 1,2-distearoyl-sn-glycero-3-phospho-(N,N-dimethyl-N-[N',N',N'-trimethyl-ethylammonio])-ethylammonium chloride
$C_{48}H_{98}ClN_2O_8P$ (897.8)
Identified by a TLC comparison with the 1,2-dipalmitoyl derivative (see substance 2)

4) 1,2-dipalmitoyl-sn-glycero-3-phospho-(N,N-dimethyl-N-[N',N'-dimethyl-ethylamino])-ethylammonium
$C_{43}H_{87}N_2O_8P$ (791.2)
calc. (%): C, 65.28; H, 11.09; N, 3.54; P, 3,92
found (%): 65.11; 11.02; 3.47; 3.88

5) 1,2-dihexadecyl-sn-glycero-3-phospho-(N,N-dimethyl-N-[N',N',N'-trimethyl-ethylammonio])-ethylammonium chloride
$C_{44}H_{94}ClN_2O_6P$ (813.7)
Identified by a TLC comparison with the 1,2-dipalmitoyl derivative (see substance 2)

6) 1,2-dioleyl-rac-glycero-3-phospho-(N,N-dimethyl-N-[N',N',N'-trimethyl-ethylamino])-ethylammonium chloride

$C_{48}H_{98}ClN_2O_6P$ (865.8)

calc. (%):	C, 66.59;	H, 11.41;	N, 3.24;	P, 3.58
found (%):	66.41;	11.35;	3.19;	3.45

7) 1,3-dihexadecyl-glycero-2-phospho-(N,N-dimethyl-N-[N',N',N'-trimethyl-ethylammonio])-ethylammonium chloride

$C_{44}H_{94}ClN_2O_6P$ (813.7)

calc. (%):	C, 64.95;	H, 11.65;	N, 3.44;	P, 3.81
found (%):	64.79;	11.59;	3.42;	3.69

8) 1,3-dioleoyl-rac-glycero-3-phospho-(N,N-dimethyl-N-[N',N',N'-trimethylamino])-ethylammonium chloride

calc. (%):	C, 66.59;	H, 11.41;	N, 3.24;	P, 3.58
found (%):	66.55;	11.40;	3.11;	3.55

Production of empty liposomes according to the present invention:

The liposomes PPGPC/N^+/chol (50/10/40) listed in Table 1 are produced.

PPGPC (5 mmol), 1,2-dihexadecyl-rac-glycero-3-phospho-(N,N-dimethyl-N-[N',N',N'-trimethylethyl-amino]-ethyl-ammonium chloride (1 mmol) and cholesterol (4 mmol) are dissolved in 50 ml CH_2Cl_2 or 50 ml $CHCl_3$ while heating in order to achieve a homogeneous mixing of the three components. The solvent is removed in a vacuum and 250 ml aqueous buffer solution (all solutions which can be used physiologically can be employed) is added to the finely dispersed lipid film. Subsequently the mixture is kept for 60 minutes at 50°C while rotating slowly

(generally about 5°C above the main transition temperature of the lipids).

The pre-heated lipid suspension is then transferred to the pressure chamber of a French press (Amico Company, Silver Spring, USA). The French press consists of a hydraulic press and a standard pressure chamber made of steel with a maximum filling volume of 40 ml. After closing the pressure chamber, the pressure is increased to 20000 psi and the liposome dispersion is pressed through a narrow outlet under constant pressure. The process is repeated at least three times. After centrifuging the liposome dispersion (Sorvall RC-5B: 5°C, 30 minutes at 27000 g) the supernatant is removed from the sediment. It contains the liposomes which are then available for the various experiments.

Production of liposomes according to the present invention with a water-soluble active substance which it is intended to transport to the liver.

PPGPC (5 mmol), 1,2-dihexadecyl-rac-glycero-3-phospho-(N,N-dimethyl-[N',N',N'-trimethylethyl-amino]-ethyl-ammonium chloride (1 mmol) and cholesterol (4 mmol) are dissolved in 50 ml CH_2Cl_2 or 50 ml $CHCl_3$ while heating in order to achieve a homogeneous mixing of the three components. The solvent is removed in a vacuum and 250 ml aqueous buffer solution (all solutions which can be used physiologically can be employed) is added to the finely dispersed lipid film. Subsequently the mixture is kept for 60 minutes at 50°C while rotating slowly (generally about 5°C above the main transition temperature of the lipids). The pre-heated lipid suspension is then transferred to the pressure chamber of a French press (Amico Company, Silver Spring, USA).

The French press consists of a hydraulic press and a standard pressure chamber made of steel with a maximum filling volume of 40 ml. After closing the pressure chamber, the pressure is increased to 20000 psi and the liposome dispersion is pressed through a narrow outlet under constant pressure. The process is repeated at least three times. After centrifuging the liposome dispersion (Sorvall RC-5B: 5°C, 30 minutes at 27000 g) the supernatant is removed from the sediment. It contains the liposomes which are then available for the various experiments.

50 ml of a 0.9 % solution of sodium chloride containing MAF (macrophage activating factor) at a protein concentration of 70 µg/ml is added to this lipid suspension. This mixture is treated in the pressure chamber in a similar manner to the production of the empty liposomes. After subsequent centrifugation, the supernatant is removed from the sediment and subjected to gel chromatography on Sepharose Cl-4B (LKB Pharmacia, length of the gel bed 70 cm). By this means MAF which is not enclosed in the liposomes according to the present invention is neatly separated from enclosed MAF. The latter requires a substantially smaller elution volume than the non-enclosed MAF (MW 30000) because of the size of the liposomes. The liposomes are concentrated for use and sterile filtered.

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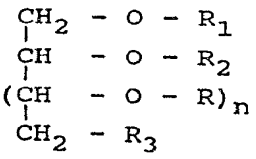
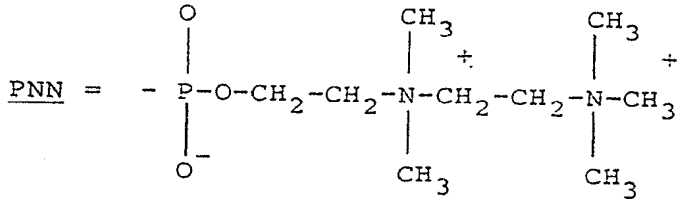
Claims

1. Liposomes
w h e r e i n
they contain at least 1 mol-% of a compound of the general formula (I) with an excess positive charge



(I)

in which
R_1 denotes alkoyl or alkyl each with 14 to 18 C atoms, oleoyl or oleyl,
R_2 denotes the group PNN



R_3 = -O-R_1, -O-PNN, -O-Gly, NH_3^+, $NH_2CH_3^+$, $NH(CH_3)_2^+$ or $N(CH_3)_3^+$,
R has one of the meanings stated for R_1, R_2 or R_3 and
n denotes a whole number from 0 to 3 provided that the molecule contains one of the said groups with a positive charge.

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2. Liposomes as claimed in claim 1,
w h e r e i n
n in formula (I) is 0.

3. Liposomes as claimed in claim 1 or 2
w h e r e i n
they contain 1 to 30 mol-% of a compound of the formula (I).

4. Liposomes as claimed in one of the claims 1 to 3,
w h e r e i n
they contain 5 to 15 mol-% of a compound of the formula (I).

5. Pharmaceutical preparation for the treatment of liver diseases containing one or several active substances which are to be transported to the liver and enclosed in liposomes,
w h e r e i n
the liposomes contain at least 1 mol-% of compounds having the general formula (I) with an excess positive charge as claimed in claim 1 in which R_1, R_2, R_3, R and n have the meaning stated in claim 1.

6. Pharmaceutical preparation as claimed in claim 5,
w h e r e i n
n in formula (I) is 0.

7. Pharmaceutical preparation as claimed in claim 5 or 6,
w h e r e i n
the liposomes contain 1 to 30 mol-% of a compound of the formula (I).

8. Pharmaceutical preparation as claimed in one of the claims 5 - 7,
w h e r e i n
the liposomes contain 5 to 15 mol-% of a compound of the formula (I).

9. Use of a compound having the general formula (I) stated in claim 1 in which R_1, R_2, R_3, R and n have the meanings stated in claim 1 or 2 for the production of a pharmaceutical agent for the treatment of diseases in the liver region.

10. Process for the production of liposomes as claimed in one of the claims 1 to 4,
w h e r e i n
at least 1 mol-% of a compound having the general formula (I) together with the other liposome components which are in an amount which together with the compound of the general formula (I) totals 100 mol-% are converted into a lipid suspension, the lipid suspension is pre-heated and the lipid suspension pre-heated in this manner is then converted in a well-known manner into liposomes by pressing and centrifuging.

11. Process for the production of a pharmaceutical preparation as claimed in one of the claims 5 to 8,
w h e r e i n
one uses the process as claimed in claim 10 for the production of the liposomes according to the invention which contain at least 1 mol-% of a compound having the general formula (I) and in order to enclose water-insoluble active substances the active substance is dissolved together with the

lipids and in order to enclose water-soluble active substances an aqueous buffer solution containing the water-soluble active substance is added to the lipid film.

SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente